improbable. All that we can conclude is that the embryo contains the different protein substances described, together with nucleic acid, and that these may unite to form a number of different compounds according to the conditions which prevail at any given time.

THE PROTEIDS OF THE EGG YOLK.

10

By Thomas B. Osborne and George F. Campbell.

The yolks of a large number of freshly laid hen's eggs were broken up by straining through a sieve and mixed with an equal volume of saturated pure sodium chloride brine. A somewhat turbid solution resulted, which was shaken out with about one-third its volume of ether, containing a little alcohol. After standing one night, a clear reddish ethereal layer separated, leaving the pale yellow aqueous solution almost clear. After shaking out a second time with ether the aqueous solution was dialyzed for forty-eight hours, whereupon a large quantity of proteid separated in spheroids which united to a salvy mass. This was freed, as far as possible, from the liquid, by draining on filters, redissolved in 10 p.c. brine and its solution dialyzed for three days. From the semi-solid mass thus obtained the liquid was decanted and the precipitate dissolved in 10 p.c. brine. A little transparent gummy substance (lecithin?) remained undissolved which rendered filtration very difficult. By filtering under considerable pressure on a thick layer of paper pulp about 700 cc. of *perfectly clear* filtrate, *a*, and 2,000 cc. of very nearly clear filtrate, *b*, were obtained. The latter, *b*, was dialyzed for four days, when the large deposit was filtered out and treated with about a liter of salt solution. A gelatinous, almost pasty mixture resulted, which was shaken out with ether. The two liquids at once separated, the ethereal being clear and strongly yellow in color and the aqueous almost perfectly clear and not at all gummy. Shaken out three times more with ether, this aqueous solution became suddenly opaque and gelatinous. The mass was dialyzed free from chlorides, the solid deposit was washed with alcohol and with ether and dried over sulphuric acid, giving preparation 1, weighing 60 grams. The *clear* solution, *a* (700 cc.), was diluted with three volumes of distilled water, and allowed to stand over night at a temperature of 6°. The proteid, which had separated on

dilution, soon settled as a clear, transparent layer, from which the clear solution, *c*, was decanted completely. The proteid was readily and completely soluble in 10 p. c. brine to a perfectly clear pale yellow solution, which, when dialyzed until wholly free from chlorides, yielded the proteid in spheroids that subsequently united to a semi-solid mass. The latter was filtered out, washed thoroughly with water and with alcohol, and dried over sulphuric acid, giving 32.1 grams of preparation **2**. The solution, *c* (decanted from the precipitate produced by dilution that yielded **2**), was treated with 1,000 cc. more water. This caused a precipitate which within two hours formed a semi-fluid deposit. From this the solution *d* was decanted, and the deposit dissolved in brine yielding a perfectly clear solution, which was dialyzed until free from chlorides.

The proteid thus precipitated was filtered out, washed with absolute alcohol as long as anything could be removed thereby and then dried over sulphuric acid, giving preparation **3**, weighing 16.5 grams.

The solution, *d*, was dialyzed free from chlorides, the precipitated spheroids were filtered out, washed with absolute alcohol and dried over sulphuric acid, making 5 grams of preparation **4**.

The solution, page 339, filtered after forty-eight hours' dialysis from the substance that yielded the foregoing preparations, was further dialyzed until almost all its dissolved proteid had separated.

The precipitate thus produced was filtered out, dissolved in brine and the solution shaken out three times with ether. On shaking out the third time, the solution suddenly changed to an opaque jelly. This was then placed in a dialyzer, and when all the salt had been removed, the insoluble proteid was washed with absolute alcohol until everything soluble therein was extracted. Dried over sulphuric acid, this preparation, **5**, weighed 75 grams. All these preparations were dried at 110° and analyzed with the following results:

	1	2	3	4	5
Carbon	50.82	51.21	51.10	50.69	50.48
Hydrogen	7.11	7.07	7.21	7.06	7.11
Nitrogen	16.04	16.11	16.23	16.40	15.50
Sulphur	1.11	1.05	1.00	1.05	1.02
Phosphorus	0.91	0.81	0.79	1.20	0.96
Ash	2.37	2.49	1.56	3.23	3.19
P_2O_5 in Ash	1.65	1.79	1.14	2.30	2.15

The ash of all these preparations consisted of sodium metaphosphate containing about 70 p. c. of P_2O_5. We have therefore subtracted from the total ash its P_2O_5, the remainder representing the ash to be deducted in calculating the percentage composition of the proteid substance.

Any chlorine or sulphur that might belong to the ash would necessarily be lost during incineration.

Calculating these preparations free from ash, as thus corrected, we have:

	1	2	3	4	5
Carbon	51.18	51.56	51.31	51.16	51.00
Hydrogen	7.14	7.12	7.24	7.12	7.18
Nitrogen	16.16	16.23	16.30	16.55	15.66
Sulphur	1.12	1.06	1.00	1.06	1.03
Phosphorus	0.92	0.82	0.79	1.21	0.97
Oxygen	23.48	23.21	23.36	22.90	24.16
	100.00	100.00	100.00	100.00	100.00

1 and 5 represent the two main fractions that weighed 60 and 75 grams respectively; while 2, 3 and 4 represent fractions of 1, whose weights were respectively 32, 16.5 and 5 grams.

In composition all are nearly alike. A little more phosphorus was found in 4 than in the other preparations, probably because a larger proportion of some phosphorus-containing acid was combined with the protein of this final, very soluble fraction, which formed less than 10 p. c. of the total vitellin.

We have not yet succeeded in preparing this phosphorized acid free from proteid. The body, which we have thus prepared from egg yolk and analyzed, is not present, as such, in the egg, for the proteid substances of the yolk are readily and wholly soluble in salt solution, whereas all these preparations are entirely insoluble in salt solution. Insolubility in the cases of 2, 3 and 4 was caused by washing with alcohol, which at the same time removed much lecithin. Lecithin was not present as an admixture, but was chemically combined with the proteid, forming a compound soluble in saline solutions and having the properties of globulin, as is shown by the following experiments.

A. The alcoholic washings from the three successive fractions, 2, 3 and 4, were evaporated and left residues of crude

lecithin weighing 6.4796, 3,5913 and 1,3150 grams respectively. Adding these quantities to the weights of the corresponding fractions dried at 110°, we have the amounts of lecithin-protein compound originally composing them. These contained 18.00, 19.4 and 22.23 p. c. of lecithin respectively.

B. The yolks of two eggs were directly extracted with ether until practically nothing more was removed. The residual matter was then, as far as possible, dissolved in 10 p. c. salt solution, filtered perfectly clear and the solution diluted with water until an abundant precipitate separated. This was filtered out, dissolved in salt solution and filtered perfectly clear. This solution and that filtered from the precipitate previously produced by diluting with water were separately dialyzed.

The proteid precipitates thus obtained were filtered out and washed with water and alcohol. The part precipitated by dilution was found to contain 17.5 p. c. of lecithin, that which remained in the diluted brine, 22.3 p. c.—results in pretty close accord with those already stated.

C. Part of a large quantity of yolk vitellin which had suddenly become insoluble on shaking with ether, was thoroughly washed with water and then completely extracted with alcohol until all the lecithin, equal to 13.31 p. c., was removed. The solution, from which the above large quantity of insoluble proteid had originally separated, still contained a little protein matter and was therefore saturated with ammonium sulphate and the salt solution of the precipitate so produced was dialyzed. The substance which then separated in spheroids, after thorough washing with water, was still readily soluble in salt solution, but when washed with alcohol became insoluble and yielded to the alcohol 24.2 p. c. of lecithin.

Hoppe-Seyler considered this lecithin to be chemically combined with the proteid, with which view our experience is in full harmony. It is not possible that such large quantities of lecithin are simply admixed with the protein matter, for were this the case it could be readily removed by ether. Furthermore we could not dissolve a mixture of globulin and lecithin in brine and obtain a clear solution easy to filter. That the proteid should unite with lecithin is to be expected, since protein readily combines with acids. We must, accordingly, consider the protein of egg yolk to be largely, if not wholly, a lecithin

compound which, dissolves in salt solution, and behaves like a globulin.

Saline extracts of egg yolk, like those of plant-seeds, contain, according to circumstances, mixtures of compounds of the protein molecule with several different numbers of molecules of lecithin, of which the more soluble compounds contain the larger number of acid molecules.

That we find such a large proportion of lecithin in these compounds is accounted for by its great molecular weight. If the molecular weight of the protein were 15,000,* its compound with four molecules of lecithin would contain over 17 p. c. of the latter.

Although we are not yet in a position to distinguish between these several compounds, it is nevertheless now necessary to make a distinction between the vitellin as it exists in the yolk, combined with lecithin, and the insoluble substance free from lecithin, which we have prepared and analyzed. As the designation vitellin has generally, if not always, been understood to apply to a protein substance, we suggest that this term be henceforth reserved for the protein, which in egg-yolk is united to lecithin and not to the compounds formed by their union, which may more properly be called lecithin-vitellin.

These considerations raise the question, are the preparations analyzed, vitellin, as defined above, or are they compounds of this protein with some other, at present unknown substance. Since the preparations yield paranuclein on digestion with pepsin, it appears highly probable that they contain paranucleic acid, but in less proportion than occurs in paranuclein.

To test this hypothesis we treated 10 grams of preparation **5** with 100 cc. of $N/_{10}$ KOH solution, and after standing some time added enough 0.4 p. c. hydrochloric acid to neutralize the alkali and give an excess of acid equal to 0.2 p. c. of the solution. Pepsin was then intermixed and the solution digested at 40° for forty hours. After some time a voluminous precipitate separated from the nearly clear liquid. This was filtered out, washed thoroughly with water and mixed with 45 cc. $N/_{10}$ KOH solution, which dissolved the precipitate and just neu-

*We have pointed out in a former paper, Jour. Am. Chem. Soc., XXI, 486, the reasons which make it probable that the weight of the protein molecule may be about 15,000.

tralized its acid reaction to phenolphthalein. This solution was filtered perfectly clear and 45 cc. of $N/_{10}$ HCl were added, which threw out the paranuclein as a voluminous gelatinous precipitate. This was filtered out, washed thoroughly with water and with alcohol, and dried over sulphuric acid. This preparation, **6**, weighed 2.38 grams. Another preparation of paranuclein was made by suspending 50 grams of preparation **5** in 2/10 p. c. hydrochloric acid, adding pepsin and, after digesting for twenty-four hours, adding more acid and pepsin and continuing the digestion twenty-four hours longer. The insoluble paranuclein was then filtered out, washed thoroughly with water and with alcohol, and dried over sulphuric acid. It weighed 15.7 grams. Preparation **7**.

The yolks of 120 eggs were mixed with an equal volume of saturated sodium chloride brine and the mixture was shaken with ether containing a little alcohol. A perfectly clear red-yellow ether-layer and a clear pale yellow aqueous layer soon separated. The ether was drawn off and the aqueous solution again shaken with ether, which caused a part of the proteid to become insoluble, rendering the solution opaque and gelatinous. After the ether had separated, the aqueous solution was dialyzed until free from chlorides, when it was filtered and the very voluminous precipitate was suspended in four liters of 0.2 p. c. hydrochloric acid and digested with 3.0 grams of pepsin. After twenty-four hours the solution was decanted from a large deposit of insoluble matter, the latter was mixed with about one-third its volume of 0.4 p. c. hydrochloric acid and the digestion continued forty-eight hours longer, whereby the amount of insoluble matter was much reduced. The latter was then filtered out and repeatedly extracted with alcohol until the evaporated alcoholic washings left no residue. This required a large quantity of alcohol and more than a week of time. The residue, air dried, weighed 32 grams and formed preparation **8**.

These preparations were then dried at 110° and analyzed, with results as follows:

COMPOSITION OF PARANUCLEIN.

	6	7	8
Carbon	46.69	47.72	44.48
Hydrogen	6.77	6.80	6.52
Nitrogen	14.66	14.64	14.34
Sulphur	0.86	0.94	0.82
Phosphorus	3.29	2.52	4.19
Ash	1.89	5.01	3.43
P_2O_5 in Ash	0.83	2.47	1.61

Assuming that the yolk proteid and the paranuclein are both compounds of paranucleic acid, we should be able to calculate the composition of the protein substance in our preparations if we knew the composition of this acid. Unfortunately we do not know this acid in the free state.

Liebermann and others have thought that paranucleic acid is identical with metaphosphoric acid, but we think it extremely improbable that metaphosphoric acid can exist in animal tissues.

In seeking to harmonize the analyses of paranuclein with those of the paranucleoproteid we find that they are brought into more or less accordance by reckoning them free from ash and from certain phosphoric acid radicals. Assuming that the hydrogen of the phosphoric acid is replaced by protein, we have "corrected" our analyses for the following phosphoric acids, viz.: HPO_3, H_3PO_4, $H_8P_2O_9$ and H_5PO_5 by subtracting from the ash its P_2O_5 and to the remainder (the bases) adding the phosphorus and oxygen of the respective acids and, after deducting their sum from 100, recalculating these remainders to percentage statements.

Corrected for PO_3 we find no satisfactory agreement, as is shown by the following figures obtained by thus correcting the analyses of preparations 2 and 6.

	2	6
Carbon	52.68	50.86
Hydrogen	7.28	7.37
Nitrogen	16.57	15.97
Sulphur	1.08	0.94
Oxygen	22.39	24.86
	100.00	100.00

Correcting for the other three acids, however, we find a close agreement between all the analyses of the preceding preparations as is seen in the following tables:

COMPOSITION OF THE PARANUCLEOPROTEID AND THE PARANUCLEIN CALCULATED FREE FROM PO_4 AND ASH.

	Paranucleoproteid.					Paranuclein.		
	1	2	3	4	5	6	7	8
Carbon	52.67	52.89	52.59	53.14	52.57	52.54	53.17	52.12
Hydrogen...	7.37	7.30	7.42	7.40	7.40	7.60	7.57	7.64
Nitrogen....	16.62	16.64	16.70	17.19	16.11	16.50	16.31	16.80
Sulphur.....	1.15	1.09	1.03	1.10	1.06	0.96	1.05	0.96
Oxygen.....	22.19	22.08	22.26	21.17	22.86	22.40	21.90	22.48
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

The agreement of these figures makes it plain that the paranuclein consists of one or more compounds of the protein (vitellin) containing a larger proportion of the same acid that exists in the paranucleoproteid from which it originated. These protein compounds with paranucleic acid are therefore analogous to those with the nucleic acid which we have obtained from the wheat embryo and described in the preceding paper.

If, as many seem to think, the paranucleic acid is not in fact H_3PO_4, it may be found to be either $H_8P_2O_9$, or H_5PO_5. These acids differ in composition so little from H_3PO_4 that we find nearly or quite as close agreement when the analyses are calculated free from either of them, as the following table shows, in which is given the result of calculating the analyses free from P_2O_9 and ash.

COMPOSITION OF THE PARANUCLEOPROTEID AND THE PARANUCLEIN CALCULATED FREE FROM P_2O_9 AND ASH.

	Paranucleoproteid.					Paranuclein.		
	1	2	3	4	5	6	7	8
Carbon	52.79	53.00	52.70	53.31	52.71	53.05	53.56	52.78
Hydrogen...	7.39	7.32	7.44	7.42	7.42	7.69	7.63	7.74
Nitrogen....	16.66	16.67	16.74	17.25	16.19	16.66	16.43	17.02
Sulphur.....	1.15	1.08	1.03	1.10	1.06	0.98	1.05	0.97
Oxygen.....	22.01	21.93	22.09	20.90	22.62	21.62	21.33	21.49
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Considering the great differences in phosphorus-content of these preparations, the striking agreement of the analyses thus calculated makes probable that the acid combined with the protein has nearly the composition H_3PO_4 and contains little, if any organic matter, or else contains an organic radical, whose composition is very nearly like that of the protein itself.

It may possibly be a methyl or ethyl phosphoric acid or some other quite simple organic phosphoric acid, but probably not so complex an acid as glycerophosphoric, as we have found a wide difference between the analyses when corrected for this acid.

It seems probable in view of these facts that further study will lead to the isolation and positive identification of this acid.

Conclusion.

Sodium chloride solutions dissolve from egg yolk a large amount of protein matter which has the properties of a globulin, being precipitated by diluting or dialyzing its solutions.

The substance soluble in salt solution consists of a mixture of compounds of protein matter with lecithin. Preparations of these compounds contain from 15 to 30 p. c. of lecithin. The more soluble products obtained by fractional precipitation contain larger proportions of lecithin than the less soluble, that is, they are more acid compounds. These compounds might well be called lecithin-nucleo-vitellin.

The lecithin thus combined is not removed by ether, but readily by alcohol. The insoluble lecithin-free proteid, obtained by treating the lecithin compounds with alcohol, has a constant composition when obtained from successive fractional precipitations of the lecithin compound. The following is the average of five accordant analyses representing fractional precipitations of the substance:

Composition of Nucleo-Vitellin.

Carbon	51.24
Hydrogen	7.16
Nitrogen	16.38
Sulphur	1.04
Phosphorus	0.94
Oxygen	23.24
	100.00

This substance on digesting with pepsin yields paranuclein of variable composition. When the analyses of the nucleovitellin and the paranuclein are calculated free from phosphoric acid H_3PO_4, possibly identical with "paranucleic acid," the composition found for the organic part of all of these preparations is so nearly the same as to show that the proteid and the nuclein are both compounds of one and the same proteid body, vitellin, with a phosphoric acid, possibly H_3PO_4, $H_8P_2O_9$, or some very simple organo-phosphoric acid. The composition of the organic part of the preparations calculated free from H_3PO_4 gives as the average for eight preparations of the paranucleoproteid and the paranuclein the following figures:

COMPOSITION OF VITELLIN.

Carbon	52.71
Hydrogen	7.46
Nitrogen	16.64
Sulphur	1.05
Oxygen	22.14
	100.00

11 THE PROTEIN CONSTITUENTS OF EGG WHITE.

BY THOMAS B. OSBORNE AND GEORGE F. CAMPBELL.

A recent paper by the writer (Report of this Station for 1898 and Jour. Am. Chem. Soc. **21**, 486) gave an account of preparations of crystallized egg albumin which justified the conclusion that with the substance commonly called ovalbumin there is associated one or more other protein bodies, the properties of which were not definitely ascertained.

We have since repeated this work on a larger scale and have not only confirmed the former observations, but have obtained much additional information respecting these other protein bodies.

FRACTIONAL PRECIPITATION OF EGG WHITE.

Six liters of the whites of freshly laid eggs were gradually and carefully mixed with an equal volume of saturated ammonium sulphate solution and formed the precipitate A, which was filtered off.